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### *The Nociceptive Withdrawal Reflex*

Jure, Fabricio Ariel

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# **PROTECTIVE MECHANISMS IN LOWER LIMB TO NOXIOUS STIMULI**

THE NOCICEPTIVE WITHDRAWAL REFLEX

**BY  
FABRICIO ARIEL JURE**

DISSERTATION SUBMITTED 2020



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Fabricio Ariel Jure



**AALBORG UNIVERSITY**  
DENMARK

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## CV

Fabricio Ariel Jure was born in San Salvador de Jujuy, Argentina in 1987. In 2013, he obtained his degree as Bioengineer at the Faculty of Engineering of the National University of Entre Ríos (FI-UNER), Argentina. After working as product engineer and project manager he traveled to Denmark and began his doctoral studies at Aalborg University, in 2015. As a Ph.D. fellow in the Integrative Neuroscience research group at SMI™, Department of Health Science and Technology (HST), Faculty of Medicine, he was involved in educational and research activities. His main areas of research involves the acquisition, analysis, and interpretation of electrophysiological signals with focus on electromyography and electroencephalography, biomechanical patterns and neurophysiology in the study of nociception and pain.





# ENGLISH SUMMARY

The protective mechanisms are part of the vital strategies developed for survival. Among the large range of innate protective reactions, the nociceptive withdrawal reflex (NWR) is a protective somatic mechanism that has been widely investigated. Due to the polysynaptic characteristic of this mechanism, the NWR is susceptible to several modulatory sources. In addition, numerous studies have been carried out to reach a better understanding of the nociceptive system and its dynamic spinal and supraspinal modulation. Limbs are open kinematic chains, encompassing multiple segments, however the description of the NWRs has been focused on isolated reactions of individual muscles or, occasionally, for a pair of agonist-antagonist muscles. As a result, current knowledge still lacks a comprehensive understanding of the entire kinetic chain involved in the defensive reaction, which may lead to a better insight on how the nervous system manages to efficiently execute motor reactions initiated by sensory information.

In this Ph.D. project, the main aim was to describe the lower limb NWR considering a combined activation of several muscles acting across several joints in the stimulated limb. The present work was driven by the hypothesis that the central nervous system (CNS) is able to modulate each group of muscles differentially, taking into account the relative position of the limb and the relevance and the role of the muscles in the lower kinetic chain.

To this purpose, three different experimental studies with healthy participants in recumbent position, were designed. By means of surface electromyographic recordings and muscle synergy analysis, a thorough characterization was performed, and the NWR was analyzed and described under different central and peripheral modulations.

Results from the first study highlight the complexity of the CNS in coordinating specific activations of the different actuators of the kinetic chain, under modulation by subtle cognitive factors. This complex muscle coordination was further characterized in the second study, describing the presence of shared neural drives and relative muscle contributions that synchronized and optimally activated the actuators involved in the protective reaction due to changes of the stimulus characteristics. Finally, the third study underlined the

different adjustments done by the CNS to tune and regulate the activation of each of the muscles involved in the protective reaction, following a certain hierarchy in the kinetic chain, while dealing with heterotopic concomitant tonic pain.

In conclusion, the present work presents the NWR as a complex integral protective reaction subjected to different modulatory mechanisms, in which the CNS tunes and controls the various actuators hierarchically across joints of the kinetic chain. Implications of this work could lead to a better use of the NWR as a biomarker to explain further spinal and supraspinal processing.

# DANSK RESUME

Beskyttende mekanismer er en del af de vitale strategier for overlevelse. I den lange række af instinktive beskyttende reaktioner findes den nociceptive afværgerefleks (nociceptive withdrawal reflex, NWR). NWR er en beskyttende somatisk mekanisme, som er beskrevet igennem omfattende forskning. På grund af den polysynaptiske karakteristik i refleksbuen er NWR følsom over for adskillige modulatoriske kilder. Der er udført omfattende forskning for at opnå en bedre forståelse af det nociceptive system og dets dynamiske, spinale og supraspinale modulation. Lemmer er biomekanisk beskrevet som åbne kinematiske kæder, der omfatter adskillige segmenter. Beskrivelsen af de nociceptive afværgereflekser har været fokuseret på isolerede reaktioner for individuelle muskler eller lejlighedsvis for par af agonist-antagonist muskler. Derfor mangler der stadig en overordnet forståelse af den fulde kinetiske kæde, der er involveret i forsvarsreaktionen, hvilket kunne lede til en forbedret indsigt i, hvordan nervesystemet effektivt udfører motoriske reaktioner, der er initieret af sensorisk information.

Målet med denne ph.d.-afhandling var at beskrive NWR i de nedre lemmer under hensyntagen til en kombineret aktivering af adskillige muskler, der agerer på tværs af leddene i det stimulerede ben. Hypotesen, der ligger til grund for studierne, var baseret på, at centralnervesystemet (CNS) er i stand til at modulere hver gruppe af muskler forskelligt under hensyntagen til den relative position af legedelen samt relevansen og rollen af musklerne i den nedre kinetiske kæde.

Til at belyse dette formål blev der udført tre forskellige eksperimentelle studier med raske forsøgspersoner i liggende position. Ved hjælp af elektromyografiske målinger og muskelsynergianalyse blev der udført en grundig karakterisering, og NWR blev analyseret og beskrevet under forskellige centrale og perifere modulationer.

Resultaterne fra det første studie fremhæver kompleksiteten i centralnervesystemets koordination af specifikke aktiveringer af de forskellige aktuatorer i den kinetiske kæde under diskrete kognitive faktorer. Denne komplekse muskelkoordination blev endvidere beskrevet i det andet studie, som beskrev tilstedeværelsen af delte neurale drev og relative muskelbidrag,

som synkroniserede og optimalt aktiverede de aktuatorer, der var involveret i den beskyttende reaktion som følge af ændringer i stimuluskarakteristikken. Endelig viste det tredje studie de forskellige justeringer, som CNS udfører for at afstemme og regulere aktiveringen af hver af de muskler, der er involveret i den beskyttende handling på basis af et bestemt hierarki i den kinetiske kæde under en heteotopisk samtidig smerte.

Afslutningsvis præsenterer afhandlingen NWR som en kompleks sammenhængende og beskyttende reaktion, der er underkastet forskellige modulatoriske mekanismer, hvor CNS hierarkisk afstemmer og kontrollerer de forskellige aktuatorer på tværs af leddene i den kinetiske kæde. Disse resultater kan betyde en bedre udnyttelse af NWR som en biomarkør til at forklare yderligere spinal og supraspinal bearbejdning.

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Finally to my family in Argentina - Mom, Dad, Ro and Candy - this is also for you, for the endless daily love and the constant support from the very first beginning, always managing to be very close despite the distance.

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Fabricio A. Jure



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# 1 INTRODUCTION

*"It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to changes" - Charles Darwin*

Across all species, one of the most vital strategies that had to be developed for survival is the protective system. A very basic self-preservation function of the motor system against attack, collision or harm is to protect the body from potential threats (Graziano and Cooke, 2006). In the presence of danger, simple behaviors such as attack, escape and/or withdrawal can be activated by the protective system in order to avoid the possible damage (Bradley et al., 2001). To perform these actions, the organisms need to integrate a perceptual and a motor system to identify and to quickly avoid the possible menace (Öhman and Mineka, 2001).

Naturally, humans have a large spectrum of innate protective mechanisms, ranging from simple instinctive behaviors such as reflexes, to more complex and multidimensional mechanisms like 'pain' (Wallwork et al., 2017). These protective mechanisms involve a large number of structures from cortical levels (e.g. sensory-motor cortex), passing through subcortical stages (e.g. brain stem), to further spinal and peripheral mechanisms (Graziano and Cooke, 2006). The final output of the integration of all these structures reflects emotional and physiological aspects triggered by the surrounding threats..

In the last century, these characteristics have led researchers to investigate deeply the protective mechanisms in general. Moreover, some of these mechanisms have been proposed to be applicable in neuro-rehabilitation programs. Several studies on gait rehabilitation have evaluated the reliability of 'simple' protective mechanisms where reflexes are utilized as gait initiators (Braun et al., 1985; Duysens et al., 1990; Richard et al., 2015 see also Andersen and Spaich (2018) for a review). Alternately, other studies focused on more complex protective mechanisms like pain have been, and still are, intensely investigated, particularly those involving chronic pain (Lim et al., 2011, 2012; Vuilleumier et al., 2017), and those involving the effectiveness of pain relieving medications (Arendt-Nielsen, 2007; Fischer et al., 2017; Lelic et al., 2017).

Remarkably, one of the most reliable and objective biomarkers employed in pain assessment, is a basic protective mechanism coined the *Nociceptive Withdrawal Reflex* (NWR) (Kralj and Grobelnik, 1973; Willer, 1977).

The NWR is an involuntary spinal mechanism that intends to withdraw the affected area out from a sufficient strong noxious stimulus to protect the body from tissue damage (Andersen, 2007). Within the response, the reflex is meant to withdraw the threatened area, while preserving balance and return into the ongoing motor program. Hence, this polysynaptic reflex integrates the afferent information of peripheral sensory neurons (e.g., nociceptors), which project their connections into the dorsal horn of the spinal cord, where it is subjected to strong, dynamic descending modulatory signals and motor commands (Sandrini et al. 2005). In this process, neural mechanisms, muscular activity and biomechanical strategies are highly integrated. The complex and dynamic integration of this innate protective mechanism led to the implementation of NWR as an electrophysiological tool in animal (Schouenborg and Dickenson, 1985; Harris and Clarke, 2003; Clarke and Harris, 2004; Bence and Cleland, 2019; Lie et al., 2019) and human (Meinck et al., 1981; Emborg et al., 2009; Biurrun Manresa et al., 2013b, 2014b; Arguissain et al., 2015; Perrotta et al., 2016) studies for the evaluation of the spinal motor processing and the nociceptive system.

## 1.1 NWR CHRONICLES

Over the past decades, the description and understanding of the withdrawal response have evolved. A century ago, Sir Sherrington (1910) first described the NWR in spinalized animals as a '*flexor reflex*'. This concept described the withdrawal reflex as a stereotyped flexion of the affected limb. The flexion presumed an excitation of flexor muscles and an inhibition of extensor muscles of the ipsilateral joints, combined with an extension of the contralateral limb to maintain posture. Furthermore, Sherrington observed particular clusters, called 'receptive fields', in where stimulations within these areas might elicit identical reflex movements (Sherrington, 1906).

In the late 50's, the first findings involving humans were published (Eklund et al., 1959; Hagbarth, 1960; Kugelberg et al., 1960) leading to a different theory of the neural control behind the 'flexor reflex'. A more thorough organization of the reflex was observed based on the responses from different stimulation sites. Changes of ankle movements from dorsiflexion to plantarflexion were recorded when stimulating the forefoot and the heel respectively (Grimby, 1963). These observations contrasted with the mentioned 'flexor reflex' concept suggesting, that the withdrawal response elicited from the skin was site-dependent. Hence, the different muscles were activated to ensure an appropriate withdrawal reaction away from the threat.

A new concept emerged, where cutaneous, joint and muscle afferents might evoke reflexes, sharing common interneuronal spinal pathways. This concept, proposed by Lundberg (1979), was coined *Flexor Reflex Afferents* (FRA).

Lundberg conceptualized the FRA idea as a system, which may be involved during active motor movements. It works as a multisensory activating system, taking the reflex as a feedback system in order to support movements instead of relying on a single afferent entity. Nevertheless the withdrawal reaction cannot be entirely explained by the FRA viewpoint, since several patterns may involve not only the flexion but also the extension of joints depending on the motor program, on the posture and/or on the stimulation site, among others.

In order to explain the withdrawal reaction including the non-flexor reflexes, an alternative model was later on proposed. From studies in rodents, Schouenborg and Kalliomäki (1990) were able to show the presence of confined reflex receptive fields (RRF) for individual hindlimb muscles in anesthetized rats. Skin stimulations within a circumscribed location elicited a distinct reflex response only for specific muscles or synergistic muscle groups, regardless of the muscle function (i.e. flexor or extensor). Subsequently, the net protective response was based on a combined activation of different muscles, rather than a stereotyped flexion response (and/or inhibition of extension response).

The suggested hypothesis introduced the '*modular organization*' concept to explain the neurophysiology of the NWR (Schouenborg et al., 1994). Each muscle or a set of synergistic muscles, associated with a RRF was termed as a '*module*', and stimulations of the skin will recruit relevant modules that will end in a *functionally appropriate withdrawal* of the threatened area (Schouenborg and Weng, 1994). Considering this, the net withdrawal reaction will be composed by the combination of different movements (i.e. flexion/extension; inversion/eversion; abduction/adduction; etc.) as result of the activation of the specifically recruited modules.

Additionally, Schouenborg and co-workers sustained the idea that the appropriate reaction patterns were imprinted on the specific receptive field of each module (Schouenborg et al., 1995). Interestingly, discreet pools of neurons in the deep lamina of the dorsal horn were identified having a corresponding '*musculotopic*' organization. These proprioceptive neurons responding to a broad range of sensory input (wide-dynamic range neurons (WDR)), were found to match with the cutaneous RRF for specific muscles suggesting the presence of individual reflex pathways running in parallel and, connecting particular cutaneous RRF to specific muscles (Schouenborg and Weng, 1994).

Even though the modular organization basis of the NWR system, where the idea of singular modules act on individual muscles, was years later supported by studies in cats (Levinsson et al., 1999a), rats (Schouenborg et al., 1994; Schouenborg, 2002), rabbits (Harris and Clarke, 2003) and humans

(Andersen et al., 1999, 2001, 2003; Sonnenborg et al., 2000, 2001), the possibility of the presence of collateral projections to close synergistic muscles cannot be dismissed (Schouenborg, 2008).

In humans, the NWR was broadly studied mainly in the lower limb (Dhondt et al., 2019). Commonly, this non-invasive method consists of a transcutaneous electrical stimulation applied on a distal part of the limb. The elicited response is measured by means of a surface electromyography (EMG) technique of the individual muscles involved. In order to quantify the EMG response, several features can be analyzed. For instance, the area under the curve, the onset latency or the root-mean square amplitude (RMS) among others, are the most common outputs analyzed (Roby-Brami and Bussel, 1987; Serrao et al., 2004; Terkelsen et al., 2004; France et al., 2009). In addition, other inferred measures, such as the RRF, have been shown to give more insights about the functional characteristics of the withdrawal reflex under diverse conditions (Neziri et al., 2009; Biurrun Manresa et al., 2011, 2013a).

Considering that the NWR is part of the vital protective system, presumably developed for survival, it is plausible to assume that this resource is triggered in an effective and efficient manner by the CNS. Then, the CNS has to take into account biomechanical (i.e. proprioceptive information) and environmental (i.e. sensory information) constraints to sharply activate the reflex system accordingly.

From a biomechanical viewpoint, the limbs are open kinematic chains enlacing different segments. Those segments are pulled by several muscles, and rotate dynamically around joints, generating different velocities and torques to carry out a variety of motor tasks (Hollerbach and Flash, 1982). The redundant number of muscles in comparison with the number of joints and the ability to produce forces with complex reliance on neural commands raise an interesting and still open question in the field, how does the CNS manage to coordinate sensory information and translate it into coordinated effective movements?(d'Avella, 2016).

## **1.2 MOTIVATION OF THE PH.D. PROJECT**

The NWR has been widely investigated during the past decades due to its particular interest as a biomarker in clinical and experimental research, and the particular employment of the NWR as a tool in rehabilitation research. Owing to the polysynaptic characteristic of this mechanism, the NWR is susceptible to several modulatory sources. There is plenty of evidence, where different intrinsic and extrinsic factors have been shown to modulate this

protective mechanism. Although those studies contributed to a better understanding of the nociceptive system and its dynamic modulation; the description of the phenomena has been focused in terms of a single, isolated, reaction of individual muscles or, in occasions, for a pair of agonist-antagonist muscles instead of in terms of a more comprehensive reaction of the entire kinetic chain.

Hence, the general purpose of this project was to describe the protective reaction considering a combined activation of several muscles acting across several joints. In particular, the present work was driven by the hypothesis that the CNS might be able to modulate each group of muscles differentially in the withdrawal reaction, taking into account the relevance and the role of the muscles in the lower kinetic chain. Moreover, the possibility of the existence of different levels of hierarchy in the motor control was explored, which would evidence a potential grade of efficiency in the neural signaling of the motor commands in the withdrawal strategies.

### **1.3 AIMS OF THE PROJECT**

The overall aim of the present Ph.D. project was 1) to characterize the withdrawal response of lower extremity kinetic chain as a complex mechanism that involves several muscles acting across several joints, 2) to characterize the modulation of the withdrawal responses due to supraspinal and spinal factors by means of muscle synergy analysis.

The NWR was used as a measure of the withdrawal response of the lower limb, elicited by electrical stimulation at the sole of the foot. The assessment of that response was analyzed altogether in terms of distal muscles (i.e. muscles that are far from the core of the body) and proximal muscles (i.e. muscles that are close to the core of the body), in order to address the following specific questions:

- 1) Do subtle cognitive factors, such as the predictability of the noxious stimulus, have an influence on the reflex pattern?
- 2) Is it sufficient to characterize the reflex pattern in terms of the analysis of a single muscle?
- 3) How do the changes in stimulation parameters, such as stimulation intensity and stimulation site, affect the reflex pattern?
- 4) Can the reflex pattern be considered as a hierarchical mechanism that can be orchestrated by shared neural drives?
- 5) Are distal and proximal muscles differentially recruited when the excitability of the nociceptive system is modulated?

These research questions were addressed in three main studies (Study I, II and III):

**Study I:** Jure, FA; Arguissain, FG; Biurrun Manresa, JA; Graven-Nielsen, T; Andersen, OK. "Stimulus predictability moderates the withdrawal strategy in response to repetitive noxious stimulation in humans". *Journal of Neurophysiology* 123(6), pp. 2201-2220. 2020.

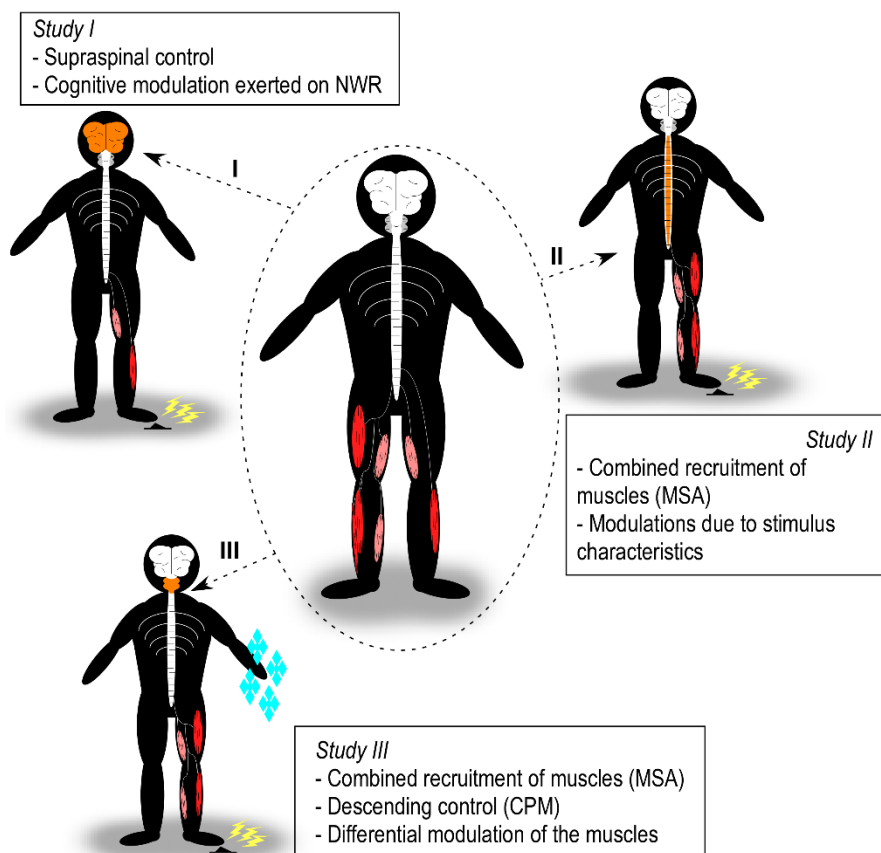
**Study II:** Jure, FA.; Arguissain FG; Wais, AA; El-Omar B; Singh Dhillon N; Spaich, EG; Andersen, OK. "Characterization of the nociceptive withdrawal reflex pattern in lower limb: A muscle synergy analysis". Submitted.

**Study III:** Jure, FA.; Arguissain, FG; Biurrun Manresa, JA; Andersen, OK. "Conditioned pain modulation affects the withdrawal reflex pattern to nociceptive stimulation in humans". *Neuroscience* 408:259-271, 2019.

## 1.4 DISSERTATION OVERVIEW

An outline of the aspects approached in each study is shown in Figure 1.

The present dissertation is organized in five chapters. The first chapter is the present introduction, which gives an overview of the protective reflex, the aims and the research questions of the thesis and the studies that addressed those questions. Chapter 2 describes the physiology behind the cognitive modulations that may influence the NWR and the main findings of Study I. Study I relates to the research questions 1 and 2. Chapter 3 introduces the muscle synergy analysis, which is a novel methodology to analyze the NWR considering a combined recruitment of muscles. This methodology was applied in Study II in order to answer the research questions 3 and 4. Chapter 4 presents the physiology behind the descending control triggered by a heterotopic conditioning pain and introduces Study III. The findings of Study III shed further insight into research question 4 and provide an answer to the research questions 5. Finally, chapter 5 summarizes the main contents of the three studies described in chapters 2, 3 and 4.



**Figure 1.** Schematic representation of the aspects explored in Studies I, II and III.





## 2 COGNITIVE MODULATION OF THE REFLEX PATTERN

As stated previously, the protective mechanisms involve several structures from cortical to spinal levels, and the net result is the integration of all the structures involved, reflecting cognitive, emotional and psychological aspects triggered by the surrounding threats.

To the reflex circuitry at the spinal cord, arrive and converge multiple ascending and descending tracts integrating the activity of several interneurons i.e. from dorsal to ventral areas, caudal to rostral segments and from peripheral and supraspinal structures. In this chapter, a description of the physiological neural substrate from cortical to spinal structures is reviewed in the light of the NWR control. In addition, particular focus is placed on the subtle psychological aspects that might affect the processing of the protective systems.

### 2.1 THE UNKNOWN

The unpredictability of the unknown in forthcoming situations, is a recurrent episode that all beings commonly face. When facing uncertainty, detecting the connection of the uncertain event with the undercurrent circumstance is essential for enabling individuals to prepare mentally and motivationally to achieve the desired outcome (Qiao et al., 2018).

From the self-protection and survival viewpoints, species had to develop the ability to predict aversive events in order to adapt the vast spectrum of innate reactions to the dynamic environment where they are involved. A diverse repertoire of adaptive behaviors, emotional states, attentional focus and/or different perceptions are caused by events that display various degrees of predictability considered in terms of certainty/uncertainty (Ploghaus et al., 2003).

From the psychological point of view, when a *certain* aversive situation is impending, it triggers an emotional state associated with *fear*. The fear emotion is a psychological state that sparks mainly two possible outcomes in every individual, which are the well-known, "*fight or flight*". When these options are unavailable, the remained action to take, is to bear with the situation by minimizing the impact (e.g. by a cognitive distraction). For instance, in studies related with the assessment of pain, fear has been proved to have an impact on its perception, leading to a hypoalgesia (Rhudy and Meagher, 2000).

On the other hand, an *uncertain* event has a different psychological effect. Uncertain events are associated with the emotional state of *anxiety*, which assumes a risk assessment behavior or, in other words, a behavioral inhibition. Under anxiety, individuals tend to increase their somatic and environmental attention (Ploghaus et al., 2003). According to several studies, anxiety has been proved to have an impact on the perception of pain by increasing its sensitivity, leading to an hyperalgesia (Rhudy and Meagher, 2000; Ploghaus et al., 2001).

Considering the above evidences, it may be relevant to investigate the neurophysiological mechanisms by which cognitive factors, such as predictability, affect the somatosensory processing and if this particular cognitive factor is able to modulate innate protective reactions like the NWR.

## 2.2 MIND OVER BODY: THE TOP-DOWN SYSTEM

Supraspinal modulation, also known as the top-down modulation system, is crucial to preserve the integrity and functional organization of the spinal cord circuitry (Dietz, 2010). In the last decades, a vast number of studies performed in spinalized animals (Schouenborg and Kalliomäki, 1990; Schouenborg et al., 1992; Levinsson et al., 1999b; Carlson et al., 2005; Bence and Cleland, 2019) and in spinal-cord injured patients (Shahani and Young, 1971; Hornby et al., 2003; Andersen et al., 2004; Knikou, 2007; Biurrun Manresa et al., 2014a; Mackey et al., 2016) have indicated a dysfunctional and, in some cases, exacerbated NWR responses after lacerations at different spinal cord levels, putting in evidence the importance of the descending drive onto the spinal cord.

The spinal nociceptive processing is highly influenced by a dynamically descending modulation from supraspinal centers. In the brainstem, the midbrain periaqueductal gray (PAG) plays an important role in the coordination between limbic forebrain areas (i.e. anterior cingulate cortex, amygdala, dorsomedial nucleus of the hypothalamus and medial prefrontal cortex) and the dorsal horn of the spinal cord (Heinricher et al., 2009). The circuitry between the PAG and the spinal cord is not direct; instead it is projected through relays at the rostral ventromedial medulla (RVM). The RVM comprises relevant structures such as the reticular formation and the raphe nucleus magnus, which are actively involved in the descending modulatory analgesia system (Ren and Dubner, 2011). Thus, the RVM sends the neuronal projections to different levels on the dorsal horn of the spinal cord.

The PAG-RVM descending system exerts not only inhibitory but also facilitatory drive on the dorsal horn (Chen and Heinricher, 2019). This bimodal nociceptive modulation is due to two different classes of RVM neurons coined as “ON-cells” (i.e. facilitatory drives) and “OFF-cells” (i.e. inhibitory drives), which have been identified to interact with primary afferent terminals at the dorsal horn (Zhang et al., 2015). The deep dorsal horn, particularly between laminae V and VI, is a nodal area in the spinal cord, where presumably the terminals from exteroceptive, proprioceptive, and corticospinal projections are conveyed and integrated (Granmo et al., 2008; Schouenborg, 2008; Tripodi et al., 2011; Koch et al., 2018). Under normal physiological conditions, these two populations of RVM-cells fire in a balanced antiphase-synchronized way (i.e. presenting fluctuations of activity and silence phases), resulting in a parallel fashion spinal modulation of the nociceptive processing (Chen and Heinricher, 2019).

However, this balance is subjected to the undercurrent behavioral situation on which the individual is placed (Heinricher et al., 2009). In this line of thinking, if the environmental surrounding changes, then the behavioral context changes accordingly and that might generate changes on the descending modulatory control. For instance, the predictability of aversive events has been proven to activate distinct cortical networks, for example, in the anticipation of the forthcoming pain (Brown et al., 2008). Activity from some cortical areas including the anterior prefrontal, inferior frontal and temporal cortices were shown to be involved in the anticipation of certain events, whereas other areas associated with attention, such as prefrontal, posterior cingulate and bilateral inferior parietal cortices were shown to be more active in anticipation of uncertain events (Peng et al., 2019). In addition, hyperalgesia driven by uncertain events can be predicted by anticipatory brain responses in the PAG (Yoshida et al., 2013). Hence, evidence seems to indicate that predictability regulates cortical responses to the anticipation and perception of pain (Peng et al., 2019).

Altogether, the described pathways, from cortical areas to spinal areas, give the neural basis through which different cognitive and/or motivational states can influence spinal transmission (Fields, 2018). Therefore, it is plausible to think that different degrees of predictability can influence not only the anticipation and perception of pain, but also can influence on more innate protective behaviors such as the NWR.

## 2.3 KNOWING MAKES THE DIFFERENCE

According to the literature, modulatory effects on the NWR, seen as isolated muscle reactions, have been reported to be induced by several cognitive and emotional states (Rhudy et al., 2005, 2013; Bjerre et al., 2011; Hubbard et al., 2011; Roy et al., 2011; Shew et al., 2011; Arsenault et al., 2013; Bartolo et al., 2013; Lannon et al., 2020).

In Study I, the experimental design was intended to assess the possible supraspinal modulations that could affect the NWRs due to different degrees of predictability of an aversive event.

In this regard, NWRs were elicited by means of repetitive noxious electrical stimulations on the sole of the foot, precisely on the arch of the plantar side of the foot under the first tarsometatarsal joint. The protective behavior expected from threats at distal parts of the lower kinetic chain presumes a withdrawal reaction of the limb towards the body's core. Particularly, noxious stimuli at the arch of the foot has been proven to induce a dorsiflexion of the ankle joint, when participants are in recumbent position.

As a first attempt to evaluate the NWR taken as a combination of several muscles acting across several joints, and considering the expected biomechanical reaction, the muscular activity of a distal (i.e. tibialis anterior (TA) - dorsiflexor of the ankle joint) and a proximal (i.e. biceps femoris (BF) - flexor of the knee joint) muscle was recorded by means of surface electromyographic techniques.

With regard to predictability of the aversive event, the characteristics of the stimulation train, such as the number of repeated stimuli in the train and the onset of the stimulus train were manipulated in order to simulate different degrees of predictability. These manipulations were meant to emulate changes in the intensity of the aversive event (i.e. stimulation train) by adding an extra stimulus on the train and, in addition, to emulate changes in the timing by indicating with a sounded cue when this event would happen. Three different conditions were randomly presented to the participants with the following characteristics:

- a) Condition 1 (high predictability): the participants were aware of the number of stimuli in each stimulation train and the onset of each stimulation train.
- b) Condition 2 (medium predictability): the participants were aware of the number of stimuli in each stimulation train, but not about the onset of each stimulation train.

- c) Condition 3 (low predictability): the participants were unaware of the number of stimuli in each stimulation train and the onset of each stimulation train.

Results from Study I suggest that supraspinal modulations triggered by different degrees of predictability of an aversive event dynamically balance the innate protective behavior of withdrawal.

In general, participants displayed a smaller 'overall NWR response' when they were completely aware of the characteristics of the aversive event (i.e. Condition 1). Furthermore, the 'overall NWR response' seems to be enhanced with less information of the aversive event, being larger under low degrees of predictability of the aversive event (i.e. Condition 2 and Condition 3). Here, the term 'overall NWR response' is meant to describe the net withdrawal response of the kinetic chain, i.e. the combined reaction of the BF and TA muscles.

The overall withdrawal behavior observed in Study I, to some extent, has been reported before. Studies investigating the possible effect of certain/uncertain events on NWRs analyzing isolated muscles, have indicated similar findings, where certain/uncertain events evoked smaller/larger NWR responses respectively (Dimitrijevic et al., 1972; Liebermann and Defrin, 2009; Quelhas Martins et al., 2015).

On the other hand, some anxiety-related studies showed controversy regarding the relationship between the reactivity of the protective system and the uncertainty of aversive events. Despite the different methodologies employed, the NWR responses observed in the BF muscle have been seen to be enhanced under threatening situations involving unpredictable painful stimulations (Willer et al., 1979; Hubbard et al., 2011; Terry et al., 2016), while no changes on the NWR thresholds of the same muscle were reported due to different levels of anxiety (French et al., 2005; Terry et al., 2012).

Assuming that the manipulation of the predictability in Study I creates a build-up effect in the level of anxiety across the experiment; the discrepancies in the literature regarding the reactivity of the protective system could be due to the fact that the assessment of the protective behavior was only performed in one muscle of the kinetic chain, concealing any modulations that may emerge for the rest of the muscles involved in the protective behavior.

When the two muscles are analyzed separately, results from Study I indicate a clear difference between their individual behaviors. For instance, under Condition 2, participants displayed facilitated BF activity which was not discernible at TA muscle. Similarly, under Condition 3 participants displayed

an enhancement of TA activity in comparison with Condition 2, which was not discernible at BF muscle. In addition, the temporal summation profile of the BF muscle was disrupted by the unpredictability of the aversive event, whereas the temporal summation profile of the TA muscle seems to follow the typical temporal summation characteristics described in the literature (Arendt-Nielsen et al., 1994; Guirimand et al., 2000).

Altogether, it is reasonable to speculate that supraspinal modulations exerted onto the spinal cord, triggered by changes associated to the predictability of an aversive event, differentially modulate the activation of the different muscles involved in the protective reaction. In this regard, as it was mentioned in section 2.2, the deep dorsal horn is presumably involved in the integration of sensory information and supraspinal commands to fine tune the motor outputs (Granmo et al., 2008). Noteworthy, Levine and collaborators (Levine et al., 2014) in their studies on animal preparations, have observed that stimulations of the intermediate premotor neurons (named Motor Synergy Encoders) activate different motor pools at the ventral horn, coordinating the necessary withdrawal reaction through the activation of different muscles (Osseward and Pfaff, 2019). Then, the net result of the withdrawal would be subjected to modulations of these interneurons and the evoked activation of the individual motor pools.

In view of the differential muscular behavior observed in Study I and considering the neurophysiological basis described in this chapter, it is likely to infer that the CNS takes advantage of the modular organization of the NWR and re-arranges the activation of the muscles following some sort of hierarchy in an effective manner where proximal muscles are recruited to exacerbate the defensive behavior, i.e. when the noxious input is sufficiently aversive a more robust withdrawal is needed.

These findings emphasize the importance of a more comprehensive description of the protective reactions through composition of the motor reaction by combined activation of several muscles acting across different joints.

## 3 COORDINATION OF THE REFLEX RESPONSE ACROSS JOINTS

One of the assumptions which this thesis is based on, is the notion that the innate protective behaviors, in general, are not simple mainly if the NWR is taken into consideration. As evidenced in chapter 2, even subtle differences that characterize a threat could lead to different net protective reactions. Moreover, the complexity of these reactions was put under spotlight by indicating different modulations on each of the analyzed actuators of the protective system. As a whole, these evidences call for a thorough analysis of this protective behavior which enlightens the dynamic mechanisms that the CNS may manage to effectively coordinate sensory information and translate it to different protective reactions.

In this chapter, an alternative and comprehensive method of analyzing the NWR involving muscles across joints based on EMG recordings is described. In particular, a characterization of the NWR pattern of the lower extremity kinetic chain is analyzed under changes in stimulus characteristics.

### 3.1 SAVING RESOURCES

The modular organization nature of the NWR was presented in section 1.1. This concept introduced by Schouenborg, establishes that the “optimal” withdrawal pattern is the consequence of the coordinated activation of diverse independent reflex modules, where each of these modules entails a set of an individual or a group of muscles and its exclusive RRF (Andersen, 2007).

However, Bizzi and collaborators have proposed a complementary modular organization of the spinal motor system. In their studies on animal preparations, they observed that cutaneous stimulations of the frog’s hindlimb elicited withdrawal reactions that can be recreated by the combined activation of particular muscle patterns evoked by microstimulations at the spinal cord (Tresch et al., 1999). In this scenario, a module is defined as a functional unit at the spinal cord, which elicits determinate motor outputs by establishing specific activation patterns of a group of muscles, referred as ‘*muscle synergies*’ (Bizzi et al., 2008).

From the motor control viewpoint, the ‘muscle synergy’ principle considers that the CNS produces a wide spectrum of complex motor movements by taking

advantage of spinal and/or supraspinal networks and by combining, in a coordinated way, small groups of muscle activations, each of them with specific amplitude balances and waveforms (Bizzi et al., 2002, 2008; Bizzi and Cheung, 2013; d'Avella et al., 2015; d'Avella, 2016; Valk et al., 2019).

The modular organization theory of the motor system suggests that the CNS preserves the resources by commanding several motor actuators with shared neural commands in an effective and an efficient manner. The synergy concept means to clarify the alleged simplifications that the CNS overcomes to manage the large quantity of independent parameters in the motor system (Banks et al., 2017).

A muscle synergy analysis assumes a reverse engineering approach. For example, if the muscle pattern is considered as the output of the CNS system, the main purpose of the analysis is to determine the different *modules* that generated the output. Each module is composed by a neural command and by specific muscle weights associated to that command. The linear combination of the modules will reconstruct the muscle pattern seen as output.

Considering the synergy concept, it is valuable to study in depth how an incoming 'noxious' afferent command, that arrives from the periphery to the CNS, leads to an orchestrated activation of the several muscles that conform the independent reflex modules proposed by Schouenborg. In this line of thinking, the muscle synergy analysis might put in evidence the possible modulations exerted onto the different muscles involved in the protective reaction, not only by identifying the neuronal commands that activate the different muscles involved, but also by indicating its individual hierarchy in the kinetic chain.

### **3.2 MUSCLE SYNERGY ANALYSIS (MSA)**

Any muscular activity involved in a movement can be considered as complex patterns generated by the CNS. In the past years, many animal and human studies, involving different motor tasks (Tresch et al., 2002, 1999; Saltiel et al., 2001; Bizzi et al., 2002; Ivanenko et al., 2003; d'Avella and Bizzi, 2005; Torres-Oviedo and Ting, 2010; Overduin et al., 2012; Ting et al., 2012; d'Avella and Lacquaniti, 2013; Wojtara et al., 2014; Sylos-Labini et al., 2020), have demonstrated that, based on the decomposition approach of the EMG activity recorded from several muscles, by combining a small set of muscle synergies it is possible to reconstruct the different muscles patterns generated in the motor task.



### 3.2.1 DATA PREPARATION

From the practical point of view, prior to the identification of the different muscle synergies, the raw EMG signals have to be pre-processed. The data processing generally involves the following sequential steps:

- 1) Band-pass filtering: this step allows to clean the raw signal from high-frequency noises, drifts and movement artifacts in order to minimize features not related to the muscle activation pattern (Torricelli et al., 2016; Kieliba et al., 2018).
- 2) Envelope extraction (i.e. rectification and low-pass filtering): the full wave rectification ensures that the signal do not average to zero and also it has been demonstrated that it increases the fire-rate information of the muscle activity (Myers et al., 2003). Then, the low-pass filter smooths the signal delineating the trend of the rectified muscle activity (Torricelli et al., 2016).
- 3) Normalization: taking into consideration that the EMG signals are recorded from different muscles, the normalization process is necessary to avoid bias in the muscle synergy extraction (Kieliba et al., 2018).

Although these pre-processing steps might be basic and trivial, they are pivotal factors in the muscle synergy analysis. The parameters chosen in these processes might have repercussions on the identification of the number of muscles synergies involved in a task and/or in the contribution balance between the muscles being analyzed (Kieliba et al., 2018).

### 3.2.2 MUSCLE SYNERGY EXTRACCION

The MSA is a computational and mathematical method proposed by Tresch and collaborators (Tresch et al., 1999) meant to decompose those complex patterns (i.e. EMG signals) into different subsets of components which generated them. This analysis aims to lower the dimensionality of a given dataset into a subset of components that can explain the majority of the variability of the primary signal (Banks et al., 2017).

This decomposition can be defined as shows Equation 1. The **EMG** matrix represents the EMG envelopes of  $m$  number of recording channels or muscles, and  $t$  time samples. The  $s$  index represents the number of subset of components to be extracted also known as *modules*. The synergistic module is formed by a neural command, denoted as **C** – referred in the

literature as *time-varying coefficients* – and by its associated values representing the degree of activation of each muscle, denoted as  $\mathbf{W}$  – referred as weighting coefficients or *muscle weights* (Clark et al., 2010). This type of muscle synergy model is known as *time-invariant muscle synergies*, where a group of muscles with specific – fixed – activation coefficients (also called synchronous muscle synergies) are assumed to be involved in the net motor activity (d’Avella, 2016).

$$\mathbf{EMG}_{m \times t} = \mathbf{W}_{m \times s} \mathbf{C}_{s \times t} + \mathbf{error} \quad (\text{Eq. 1})$$

The linear combination of these components will result in the reconstruction of the original signals and an intrinsic remaining matrix error.

The principal objective of the matrix decomposition (or matrix factorization) is to find the unknown parameters:  $s$ ,  $\mathbf{W}$  and  $\mathbf{C}$ . There are several factorization algorithms employed to identify and extract muscle synergies depending on signal-noise ratio and constraints assumptions (Torricelli et al., 2016). Factor analysis, independent component analysis and nonnegative matrix factorization (NMF), among others, are some examples of factorization algorithms used in this process (see Singh et al., 2018 for a review of the different algorithms performance).

One of the assumptions to take into consideration in the MSA, is the fact that the muscle activation is constrained to be a non-negative feature (d’Avella, 2016). From the physiological viewpoint, neural commands are meant to be positive, i.e. either a neuron fires an action potential or it remains in resting state. Due to this constraint, the most common and popularized matrix factorization method used for the muscle synergy extraction is the NMF algorithm (Rabbi et al., 2020). This algorithm is based on a gradient descent and least square techniques, uses an iterative searching method to find a set of components (i.e. to obtain  $\mathbf{W}$  and  $\mathbf{C}$  values) which adequately can explain most of the variability of the original dataset (Lee and Seung, 1999; Ting and Chvatal, 2010).

### 3.2.3 MUSCLE SYNERGY IDENTIFICATION

To assess the fidelity of the reconstructed signals, two different metrics are frequently used: ‘Variance accounted for’ (VAF) and  $R^2$  (or “centered” VAF) – equation 2. These metrics basically indicate how well the reconstructed signal explains the original signal by quantifying the data variation accounted by the reconstruction (Torricelli et al., 2016).

$$VAF = 1 - \frac{\text{sum of squared error}}{\text{total sum of squares}} \quad (\text{Eq. 2})$$

The two metrics by definition are similar. They differ on the way that the total sum of squares is calculated, VAF considers the total sum of squares with respect to zero, whereas  $R^2$  considers the calculation with respect to the mean (Singh et al., 2018). Higher values of these metrics represent a better fidelity of the reconstructed signals.

The VAF and the  $R^2$  are employed in the identification of the number of synergistic modules to be used (i.e. to obtain the  $s$  parameter in Eq. 1). For instance, by iterating the factorization algorithm from 1 to  $m$  times, a graphic representation of the metric as a function of the number of synergistic modules can be obtained.

Based on this graph, there are a several ad hoc criteria to determine how many synergistic modules are sufficient to explain the original EMG dataset. Some of the most used criteria are:

- *Best Linear Fit method*, considering the number of synergistic modules where the curve reaches a plateau, that is where the metric curve approximates to a line curve (Cheung, 2005);
- *Elbow method*, considering the number of synergistic modules at the point where a certain decrement of the slope is reached (Tresch et al., 2006);
- *Knee Point method*, using a bootstrapping technique, it considers that the number of synergistic modules will be where the increment of the number of modules produces an increment of the metric smaller than 75% of that is expected by chance (Cheung et al., 2009);
- *Threshold method*, considering the number of synergistic modules where the curve surpasses certain value of the metric, for example 90% of the metric (Torres-Oviedo, 2006).

Regardless of these identification methods, the final number of synergistic modules should be able to represent the original dataset. New modules should not change significantly the other components. Moreover, addition of more synergistic modules should take the physiological relevance to the motor task under analysis into consideration (Ting and Chvatal, 2010).

After all of these steps, the decomposition of the raw EMG signals into synergistic modules ( $s$ ) with the associated time-varying coefficients ( $\mathbf{C}$ ) and muscles weights ( $\mathbf{W}$ ) is completed, giving all the parameters defined in Eq.1.

### 3.3 DECODING THE PROTECTIVE PATTERN

Considering the modular organization of the spinal motor system, it is viable to picture the protective behavior, in terms of NWR, as a combined recruitment of different muscle synergies.

In Study II, the experimental design was intended to describe the different synergistic modules that governs the protective behavior in the lower extremity kinetic chain. In addition, the methodology applied was intended to assess the possible modulations that could affect the protective pattern. In particular, those modulations were caused by variations in the stimulus characteristics of the aversive event, for instance the intensity and the location of the threat.

In this regard, NWRs were elicited by means of electrical stimulations on the sole of the foot, while the participants were at recumbent position. The electrical stimulations were randomly delivered on four different places distributed on the sole of the foot:

- S1. Arch of the foot – under the 1st tarsometatarsal joint,
- S2. Forefoot – between the 2nd and 3rd metatarsophalangeal joint,
- S3. Lateral side of the foot – under the 5th tarsometatarsal joint,
- S4. Heel – above the plantar side of calcaneus.

Each of these sites were randomly stimulated at three different increasing intensities:

- I1. Low intensity – below pain threshold,
- I2. Mild intensity – approximately at pain threshold,
- I3. High intensity – above pain threshold.

In order to characterize the protective pattern due to the variations in the stimulus characteristics described, the NWR reactions were analyzed using a time-invariant muscle synergy approach. The analysis in Study II considered the muscle activity of five muscles of the lower limb. By means of surface EMG, these five muscles were recorded in order to describe the protective reaction across the three main joints (i.e. ankle, knee and hip joints):

- M1. Tibialis anterior (TA) - distal: dorsi-flexor and invertor of the ankle joint
- M2. Peroneus longus (PL) - distal: evtor of the foot and plantar-flexor of the ankle joint.
- M3. Gastrocnemius medialis (GM) - distal: plantar-flexor of the ankle joint and flexor of the knee joint.
- M4. Biceps femoris (BF) - proximal: flexor of the knee joint.

M5. Rectus femoris (RF) - proximal: extensor of the knee joint and flexor of the hip joint.

Results from Study II and Study III (section 4.3) suggest that the protective withdrawal behavior of the lower kinetic chain can be reconstructed by the linear combination of two synergistic modules (SM1 and SM2), in response to electrical stimulations on the sole of the foot. These modules were consistent irrespective of changes in the stimulation parameters.

The overall NWR response explained by means of synergistic modules presented two different time activation profiles associated with the specific muscle weights: SM1 is describing an early response and SM2 is describing a late response. The time activation profiles of the synergistic modules were consistent in Study II and Study III, presenting a maximum activation burst within the time-interval between 90-110 ms for SM1 and a maximum activation burst after 140 ms for SM2. In agreement with the literature, these two temporal components were previously observed in NWR responses elicited in the lower limb (Grimby, 1963; Shahani and Young, 1971; Willer, 1977; Meinck et al., 1985; Dowman, 1991). However, these previous studies outlined a controversy regarding the cutting points and the length of the two temporal components. These discrepancies could rely on the fact that the observations in the literature were based on visual inspections of the EMG signals of isolated muscles, whereas the results of Study II and Study III described the neural commands common to a group of muscles.

With regard to the effects of intensity on the overall NWR response, findings from Study II showed an enhancement of the time activation profiles with higher stimulation intensity, irrespective of the stimulation site. In this regard, previous studies have suggested that higher stimulation intensity generates larger NWR responses (Chan and Dallaire, 1989; Dowman, 1991; Andersen et al., 2001). This observation, to some extent, was expected since more aversive threats would lead to higher reactivity of the protective system.

An interesting and unexpected finding from Study II was related to the effects of stimulation site on the time activation profiles. Regardless of stimulation intensity, the area of stimulation was observed to have little influence on the time activation profiles. However, stimulations at S1 led to brisker overall NWR responses in comparison with other sites. This particular finding has been observed before in studies investigating the NWR under different postures and during locomotion (Andersen et al., 2003, 2005; Spaich et al., 2004; Richard et al., 2015). These studies have indicated that stimulation on this specific location elicits larger responses in several muscles of the lower limb. Considering this observation, it can be speculated that the arch of the foot presents different tissue properties, such as thinner skin layers and/or perhaps

different density of sensory fiber innervation, in comparison with other areas across the sole of the foot, which could explain the differences found across sites. However, results from histological preparations have rejected these speculations (Frahm et al., 2013). Thus, it is plausible to infer that the larger protective reactions observed in response to stimulations within the arch of the foot could be due to evolutionary factors (e.g. a barefoot bipedal walking) rendering less sensitive areas at the forefoot and heel; and a prevailing spinal integration at the arch, perhaps to assist other motor functions.

With regard to the muscle contributions to the synergistic modules, results from Study II and Study III suggest a clear difference on the recruitment between muscles. For instance, some muscles seem to contribute almost exclusively to only one of the modules. This was the case for TA that mainly contributed to SM1, whereas the contribution of RF was mainly to SM2. Similar observations have previously been reported, where stimulations on the sole of the foot elicited an early reflex response in TA (Sonnenborg et al. 2000a) and late reflex responses in RF (Roby-Brami and Bussel, 1987; Decchi et al., 1997). In particular, Study II and Study III indicate a preferential recruitment of muscles acting at the distal joint (i.e. ankle joint) in the early phase of the protective reaction (SM1). On the other hand, a preferential recruitment of muscles acting at proximal joints (i.e. knee and hip joints) seem to take place in the late phase of the reflex behavior (SM2), perhaps indicating a strengthening of the protective reaction. These observations suggest a time-dependent recruitment of these muscles, possibly indicating a hierarchy across the actuators of the kinetic chain. When a strong reflex response is needed, the reflex behavior might resemble a more “stereotype flexor pattern” (Sherrington, 1910).

In addition, one of the main findings from Study II was that distal muscles were exclusively stimulus site-dependent whereas proximal muscles were exclusively intensity-dependent.

The distal muscle contributions to the synergistic modules found in Study II seem to follow the modular organization nature of the NWR, where the functional - “optimal” - withdrawal pattern depended on the stimulation site. As it was stated in section 1.1, there is a vast amount of documented evidence from studies in cats (Levinsson et al., 1999a), rats (Schouenborg and Kalliomäki, 1990; Schouenborg et al., 1994; Schouenborg, 2002), rabbits (Harris and Clarke, 2003) and humans (Andersen et al., 1999, 2001, 2003; Sonnenborg et al., 2000, 2001) that support the modular organization nature of the NWR. However, it is still unclear how the modular organization is working across synergistic muscles, and thus how the net, optimal protective reaction is composed. In this regard, the observations of Study II bring information to this matter, suggesting a crucial function of distal muscles

signaling a fine-tuning of the protective pattern mainly in the beginning of the protective reaction.

Interestingly, the muscle contributions of proximal muscles observed in Study II support the findings of Study I. Results from Study II showed larger contribution of the proximal muscles due to increments on the stimulation intensity, regardless the stimulation site. Previous studies on RRF in humans, have indicated the expansion of receptive fields of these muscles due to increments on the stimulation intensity on the foot (Andersen et al., 1999; Sonnenborg et al., 2001). However, findings from Study II not only indicated larger reactions of these muscles associated to intensity but also indicated a differential recruitment of these muscles. This was observed in BF, shifting its contribution from SM2 towards SM1 with higher stimulation intensity. This shift might imply an early knee flexion allowing for a subsequent hip flexion as an effective protective behavior.

Finally, the observations in Study II provide information about common neural commands driven by the CNS, which could allow for further differential modulatory control on the recruitment of the different actuators involved in the protective behavior.





## **4 COORDINATION OF THE REFLEX RESPONSE UNDER SUPRASPINAL MODULATION**

In the previous chapters it was established that the protective behavior involves complex mechanisms integrating supraspinal and spinal structures which modulate the net protective motor response. Subtle psychological aspects can lead to a differential recruitment of the muscles involved in the withdrawal reaction (Study I), giving the idea that the CNS manage to modulate the different actuators involved in the protective behavior following a hierarchy across the different segments of the limb.

A later comprehensive characterization of the protective behavior demonstrated a coordinated activation of the muscles showing a differential degree of recruitment between the muscles acting at distal and at proximal joints, when the stimulus characteristics are changed. The degree of activation of the muscles acting at the distal joint were particularly site-dependent while the degree of activation of muscles acting at proximal joints were particularly susceptible to how much aversive the threat is (Study II). Furthermore, this comprehensive characterization indicated common neural commands that may conform part of the dynamic mechanisms by which the CNS can take advantage of and differentially modulate the different actuators of the kinetic chain.

In this chapter, a brief description of a particular behavioral phenomenon that triggers descending control onto the spinal cord by supraspinal structures is reviewed. In particular, an analysis of the NWR pattern of the lower extremity kinetic chain is presented, when the spinal excitability is affected by altered descending modulation.

### **4.1 FROM THE SPINE: ROUNDTrip TO SUPRASPINAL STRUCTURES**

The physiological basis by which supraspinal structures manage to modulate the spinal nociception is described in section 2.2. Incoming sensory information from spinal structures is transmitted to supraspinal centers which may lead to the perception of pain perception.

From the periphery, in response to an aversive noxious stimulus, the action potential travels through unmyelinated C-fibers and myelinated A $\delta$ -fibers passing through the dorsal root ganglion making synapse with a second neuron at laminae II and III, and laminae I and V at the dorsal horn, respectively (Dubin and Patapoutian, 2010). From there, a second neuron decussates into the lateral tract and travels through the anterolateral system (formed by the spinothalamic, the spinoreticular, and the spinomesencephalic tracts) to the thalamus. Then, from the thalamus a third neuron carries the information to the cortical structures, allowing the experience of the pain perception (Fields, H. L., Basbaum and Heinricher, 2006).

In its way to the thalamus, the anterolateral system extends collateral projections into several structures at the brainstem. These collateral projections reach structures at the RVM in the medulla (specifically at the reticular formation), the parabrachial nucleus at the pons, and the PAG at the midbrain. These brainstem structures are involved, directly or indirectly, in the descending modulatory system (Fields, H. L., Basbaum and Heinricher, 2006; Lockwood and Dickenson, 2020).

The descending control plays an important role as a feedback loop in the nociceptive processing, diminishing the excitability of nociceptive inputs at spinal levels. It is formed by several supraspinal structures, such as the periventricular gray matter (PVG), the PAG, the locus coeruleus, the reticular formation and the raphe nucleus magnus; and are mainly coordinated by the PAG-RVM descending system, as mentioned in section 2.2 (Benarroch, 2012; Chen and Heinricher, 2019).

In reaction to the incoming nociceptive drives from the anterolateral system arriving to cortical levels, the descending system sends projections to the dorsal horn of the spinal cord (laminae V-VI) releasing norepinephrine and serotonin, targeting a pool of interneurons in the deep dorsal horn. These interneurons, which are believed to be primarily WDR neurons (Schouenborg and Dickenson, 1985; Le Bars and Cadden, 2008), release a group of opioids reducing the neuron excitability at the dorsal horn. As result, the descending system operates like an endogenous analgesic system, which reduces/inhibits the incoming sensory information from the periphery.

## 4.2 “PAIN-INHIBITS-PAIN” MODEL

In the late 70's, animal studies carried out by Le Bars and co-workers (Le Bars et al., 1979) indicated that sufficiently strong noxious stimuli applied to heterotopic parts of the rat's body induced inhibition of the dorsal horn

neurons. The inhibition was observed, regardless of the proximity of the noxious stimulus to the excitatory receptive field under study. This phenomenon, based on the *spinal-bulbo-spinal* loop presented in section 4.1, was coined “diffuse noxious inhibitory control” (DNIC) (Villanueva and Le Bars, 1995).

The counterpart to the DNIC phenomenon in clinical and experimental human studies was years later named “Conditioned Pain Modulation” (CPM). This concept not only allows the lower brainstem-mediated loop, but also accepts further psychophysical components that might concomitantly occur in the process (Yarnitsky, 2010; Torta et al., 2019). Hence, the CPM is defined as a psychophysical paradigm where the behavioral response to a painful test stimulus (TS) is assessed during the simultaneous application of an extra – heterotopic – conditioning painful stimulation (CS).

In the past decade, numerous studies have signaled the impairment of the CPM inhibitory effects as an important biomarker of chronic pain and its further possible application as a predictor of treatments efficacy (Yarnitsky, 2010; Yarnitsky et al., 2014). Due to these findings, a vast number of experiments have been carried out, employing different modalities of TS and CS to bring more insight and to enhance the reproducibility and reliability of the CPM paradigm (see Kennedy et al., 2016 for a review).

In particular, a comprehensive review (Dhondt et al., 2019) described how the NWR has gained prominence as an objective measurement of the spinal excitability over the self-reported approach typically used on CPM paradigm. The studies comprised in the review (Dhondt et al., 2019) along with other studies (Willer et al., 1984, 1989; Le Bars et al., 1991; Terkelsen et al., 2001; Serrao et al., 2004; Biurrun Manresa et al., 2014b; Lie et al., 2019), showed that the CPM paradigm induced a descending inhibitory effect over the net withdrawal response, classically assessed in one muscle.

Considering the CPM as a dynamic test to assess the nociceptive processing mechanisms, it is pertinent to analyze the differential modulatory control that the CNS might exert on the recruitment of the different actuators involved in the protective behavior, under this paradigm.

### 4.3 “COOLING DOWN” THE PROTECTIVE REACTION

In Study III, the experimental design was intended to assess the possible supraspinal modulations that could affect the protective pattern when a conditioning tonic stimulus is applied on another body location (also referred as “heterotopic noxious conditioning stimulation”) and to characterize the differential descending modulations that might be exerted to the different actuators of the lower extremity kinetic chain.

In this regard, NWRs were elicited by means of electrical stimulations on the on the arch of the plantar side of the foot, while the participants were at recumbent position. The conditioning stimulus implemented was based on the cold pressor test (CPT), in which participants had to hold one of their hands in ice-cold water for a period of time (~3 min.).

Similar to Study II, the NWR reactions were analyzed using a time-invariant muscle synergy approach. In Study III, the analysis considered the muscle activity of four muscles of the lower limb:

- M1. Tibialis anterior (TA) - distal: dorsi-flexor and invertor of the ankle joint
- M2. Soleus (SL) - distal: plantar-flexor of the ankle joint.
- M3. Biceps femoris (BF) - proximal: flexor of the knee joint.
- M4. Rectus femoris (RF) - proximal: extensor of the knee joint and flexor of the hip joint.

The results from Study III, concerning the description of the protective reaction in terms of a linear combination of synergistic modules are in agreement with the findings of Study II, despite the fact that not all muscles recorded were the same in the two studies. Thus, as it was described in section 3.3, the protective withdrawal behavior of the lower kinetic chain can be reconstructed by the linear combination of two synergistic modules (SM1 and SM2). In addition, the muscle contributions to the synergistic modules from Study III are well corresponded with those presented in Study II.

In connection with the assessment of the protective behavior under a conditioning stimulus, in Study III the protective reaction underwent a pronounced decrement during CPT. The depression of the protective behavior caused by the descending inhibition was observed in the time activation profiles, whereas the muscle contribution to the modules seem to remain unaffected. In agreement with Study II, the fact that the muscle contribution to the modules remain unchanged supports the modular organization notion of the reflex, since the stimulation parameters were unaltered across the experimental session.

Interestingly, the inhibitory neural drive observed during CPT was unequally exerted between the two synergistic modules. In this regard, the time activation profile of SM1 presented a reduction of its activity in the time-interval between 118 ms and 156 ms, not affecting the period of maximum activity for this module (i.e. 90-110 ms). This interval could be considered as a transition zone between the two time activation profiles of the synergistic modules. Conversely, the time activation profile of SM2 presented a pronounced decrement in the main time-interval of activity for this module (from 150 ms until the end of the reflex reaction).

Previous studies have reported an inhibition of the excitability of the NWR in humans due to CPM, usually (and mostly exclusively) taking as a main outcome the assessment of the activity of BF muscle (Willer et al., 1984, 1989; Le Bars et al., 1991; Terkelsen et al., 2001; Serrao et al., 2004; Biurrun Manresa et al., 2014b). Remarkably, the NWR recordings measured at BF have been shown to present an activation burst that is comprised in the time-interval that extends across the time activation profiles of the two synergistic modules presented in Study III (Willer et al., 1989; Andersen et al., 1999).

When the individual synergistic modules were analyzed by muscle, it can be observed that the reconstructed patterns from all muscles in SM1 suffered a minor, almost negligible, depression of its reactions. However, the reconstructed patterns in SM2 showed a substantial difference between RF and the rest of the muscles; RF activity was considerably depressed during the conditioning stimulus. In addition, a reduction of approximately half of the activity was observed for the reconstructed patterns of BF and SL for SM2. However, the inhibition seen at BF muscle was likely sufficient to shift the late reaction of this muscle to a lower hierarchy of action in comparison with the earlier reaction for this muscle during the conditioning (i.e. the reconstructed pattern of SM2 for BF muscle showed lower level of activity than the reconstructed pattern of SM1).

Lastly, the combination of both synergistic modules reconstructs the final EMG activity showing alterations in the protective pattern due to the conditioning stimulus. The changes observed were mostly affecting the two most proximal joints of the lower kinetic chain, by modulating the activation of the BF and RF muscles. Notably, even though there were no substantial changes in the muscle weights nor in the early and late reconstructed patterns of BF, the conjunctive combination of the synergistic modules led to less activation of this muscle. Hence, it can be assumed that the CNS manage to coordinate the action of the different actuators of the kinetic chain by a complex combination of imperceptible changes in the excitability of the common networks.

It is worth to highlight that, to the best of the author's knowledge, there is no human study addressing the effects of descending inhibition due to CPM on other muscles in the lower limb, for example, on distal muscles. Observations from Study III suggest that the conditioning stimulus do not substantially modulate the activity of the muscles acting at the ankle joint. Similar to these observations, studies in animals have shown that under a conditioning stimulus most of the reflexes observed at hindlimb muscles of rodents were depressed except for those reflexes elicited at the plantar flexors of the digits, which were facilitated (Kalliomäki et al., 1992). Thus, in response to an aversive threat under a concomitant pain, the CNS manage to conserve some protective behaviors to diminish the possibility of acute injury while, at the same time, saving resources for a later escape in case of need (Morgan, 1999).

## 5 SYNTHESIS

The present PhD dissertation described and analyzed the withdrawal response in terms of a combined activation of several muscles working harmonically across several joints in the ipsilateral limb. Particularly, the present work characterized and outlined some of the possible strategies and modulations orchestrated by the CNS to the different group of muscles in the lower extremity kinetic chain, under different spinal and supraspinal conditions.

Throughout the work presented in this thesis, the impact of a subtle cognitive factor, such as the predictability of a forthcoming aversive event, to the withdrawal response was investigated. Study I shows that the level of certainty/uncertainty of a forthcoming aversive event, affect innate protective behaviors, not only by moderating the net result of the protecting pattern, but also by pointing out complex interactions between its actuators. These psychophysiological results, have interesting methodological implications in future clinical and experimental studies involving the protective responses. In addition, the findings of Study I pose and recognize the NWR as a joint effort of a group of muscles interacting together. These interactions lead to the concept of a differential modulation of the muscles involved in the withdrawal reaction, which was evidenced, for instance, by the disruption of the temporal summation profile in one of the two muscles analyzed. This observation was an interesting secondary outcome of the Study I. The temporal summation to repetitive stimulations paradigm did not follow the stereotyped profile that has been established in the literature, possibly opening for a deeper exploration of this phenomenon, for example considering other physiological circumstances. Finally, the Study I results prompt for a more comprehensive analysis of the withdrawal reaction that can be able to disentangle those 'hidden' modulations that might be unnoticed when tested individually.

By means of the muscle synergy concept and methodology, Study II provides a more comprehensive analysis of the protective responses, where several muscles across the main joints of the lower limb were collectively explored in response to changes associated to the stimulus parameters, like stimulus intensity and stimulus location. This analysis revealed intrinsic differences between distal and proximal muscles based on their contribution to the net protective behavior in response to changes on the stimulus characteristics. Thus, it is reasonable to infer that the actuators at different levels of the kinetic chain are differentially recruited based on a hierarchized organization of the protective system. It is proposed in this thesis that the hierarchy followed by the CNS, plausibly based on survival, is organized in a fashion where the most distal joints are the first responders to avoid the danger, escalating towards

the body core with more aversive sensory inputs. In light of the evidences presented in the Study I and the Study II, it can be conjectured that the CNS takes advantage of the spinal modular organization of the NWR and hierarchically re-arranges the activation of the different muscles in the kinetic chain, depending on the surrounding circumstances and on the severity of the threat, ending in a specific - “optimal” - protective behavior.

Furthermore, Study II and Study III outline the presence of particularly two common neural commands that may be part of the repertoire of dynamic mechanisms used by the CNS to ‘optimally’ withdraw the lower limb in recumbent position. In this regards, Study III puts in evidence those subtle integrative mechanisms to accomplish the protective reaction while dealing with a concomitant pain, highlighting the role of the descending drive in the motor control. Supraspinal structures were seen to modulate the neural commands that arrive to the dorsal horn of the spinal cord, prior to the motor pools in the spinal cord, while the intrinsic configuration of the relative muscle weights remain virtually unaltered. The subtle modulations on the neural commands may mainly influence the strength and timing of the contraction of the muscles related to more critical joints (as it was suggested by Study I and Study II), such as the knee and the hip. This is supported by the fact that the protective behavior was practically unaffected in the early phase of the reaction, whereas the late phase suffered a pronounced inhibition. Remarkably, these observations imply that the CNS manages to modulate differentially the various actuators of the kinetic chain via a top-down modulation, in agreement with Study I observations. Thus, it is plausible to indicate that the CNS targets and modulates the different actuators involved in the protective response following the hierarchy of each actuator at the different levels of the kinetic chain. In this way, the CNS has access to the different levels of the protective behavior, balancing and coordinating each of the segments of the kinetic chain in an efficient fashion to accomplish a particular reaction. This reaction is shaped according to the position of the body and the limbs (e.g. upright, active position versus recumbent position), and also according to the situational need, safeguarding the tissue and concomitantly allowing for further activations of other motor programs.

To conclude, the nociceptive withdrawal reflex in humans was analyzed and discussed as a stereotyped reaction instead of being recognized as a joint effort of a group of muscles. The withdrawal reflex was thought as a single reaction for a single muscle, or at the most as a group of agonist – antagonist muscles. The new perspective proposed and presented here considers the possibility of an exhaustive analysis of the muscle activity across the three main joints in the lower limb, which can reflect the differential modulation exerted by the CNS to the muscles and outline the complex interactions between them in the kinetic chain.



## 5.1 LIMITATIONS OF THE METHODOLOGY

The experimental studies in which the present dissertation is based, are not exempted of methodological limitations. As it was stated in section 3.2, there are several decisions which may influence the final outcome and the interpretation of the findings. Decisions regarding to the choice of the data pre-processing parameters, the selection of the computational algorithm for the decomposition and/or the election of the criteria methods for the identification of the components, are some examples of the intrinsic limitations of the muscle synergy analysis (MSA). Despite of the effort of the research community to unify and convene the best methodology to perform this analysis, there is no consensus on the choice of a 'perfect' algorithm, criteria or technique to accurately decompose and identify the different components (Steele et al., 2015; Kieliba et al., 2018; Singh et al., 2018). Nonetheless, it is important to highlight that one of the fundamental aspects to take into consideration when performing a MSA is the quantity of muscles to be recorded for the analysis. This aspect is a key factor in the decomposition and in the detection of the different synergistic modules involved in a given motor task. The analysis of a low quantity of muscles might end in a poor identification of muscles synergies due to an overestimation of the VAF (or  $R^2$ ) metric (Steele et al., 2013). However, due to biomechanical and experimental constraints it is not always possible to measure the activity of a large group of muscles. In this regard, Steele and collaborators (2013) have indicated that it is still possible to perform a synergistic analysis with a reduced number of muscles. By selecting a subset of muscles that specifically include the most dominant and large muscles involved in the motor task under observation, it can be possible to decrease the sensitivity of synergies to external constraints, improving the identification of the synergistic modules.

In Study II and Study III, the MSA was performed with a low number of muscles. The motor task under observation was an involuntary withdrawal reaction of the lower kinetic chain. This reaction assumes a withdrawal of the limb towards the body's core considering the recumbent position on which the participants were located. The reaction expected, according to the stimulation sites, involves four degrees of freedom (DoF): at the ankle joint (2 DoF: dorsiflexion/plantarflexion and inversion/eversion); at the knee joint (1 DoF: flexion/extension) and at the hip joint (1 DoF: flexion/extension). These four DoF were assessed by the recording activity of the principal muscles involved in those movements (see section 3.3 and section 4.3). The assessment of more muscles would have introduced noise to the raw dataset. For example the recording of the iliopsoas muscle (i.e. hip flexor) would have been difficult to record by means of surface EMG due to its anatomical location. Other muscles that are accessible with surface EMG like the tensor fasciae latae (i.e. hip rotator/flexor), the semitendinous (i.e. hip extensor and knee

rotator/flexor) or the peroneous brevis (i.e. ankle evor/planarflexor) would have introduced noise due to EMG crosstalk with the recorded muscles. On the other hand, the assessment of the gluteus maximum and medium (i.e. involved in the rotation, extensor/flexor and adductor/abductor of the hip) would have provided additional information on the withdrawal reaction. Furthermore, the incorporation of kinematic recordings across the three main joints would have contributed to a better description of the biomechanics, although this presents little information on the neurophysiology behind the protective reaction.

Yet, regardless of the low number of muscles recorded, the two studies arrived to the same number of synergistic modules with similar time-varying components and muscle weights.

As a final remark, it is proposed then to hypothesize a priori the number of synergistic modules that should be considered according to the biomechanical and physiological aspects of the specific motor task to be studied, in order to appropriately understand and interpret the results of the MSA (Ting and Chvatal, 2010).

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